

Western blot assay

LW Leiming Wang SYT Sophia Y. Tsai MT Ming-Jer Tsai

Updated date: Jan 28, 2021



An abbreviated version of this protocol was published in Science Advances in Apr 2020

Small-molecule inhibitor targeting orphan nuclear receptor COUP-TFII for prostate cancer treatment

DOI: 10.1126/sciadv.aaz8031

Detailed protocol

1. Cells were collected and washed with PBS, then RIPA buffer were used to get cell lysate.
2. Cell lysate supernatant was collected by centrifuge (12000rpm, 20min, 4°C), 4X laemmli buffer (biorad, 161-0747) was added.
3. 4-15% mini-protean TGX gel (biorad, 4561086) was used for electrophoresis (Marker: thermo 26634).
4. Nitrocellular membrane (biorad, 162-0112) was used for membrane transfer (250mA, 90min)
5. Membrane blocking: 5% non-fat milk was used (RT, 30min)
6. Primary antibody (1:1000 dilution in PBST with 5%BSA) was incubated in cool room overnight.
7. Washing: PBST was used for washing (3 X 10min)
8. Secondary antibody was incubated at RT for 1 hour.
9. Pierce ECL western blot substrate was used for staining.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wang, L. , Tsai, S. Y. and Tsai, M. (2021). Western blot assay. Bio-protocol Preprint. bio-protocol.org/prep788.
2. Wang, L., Cheng, C., Qin, J., Xu, M., Kao, C., Shi, J., You, E., Gong, W., Rosa, L. P., Chase, P., Scampavia, L., Madoux, F., Spicer, T., Hodder, P., Xu, H. E., Tsai, S. Y. and Tsai, M.(2020). Small-molecule inhibitor targeting orphan nuclear receptor COUP-TFII for prostate cancer treatment . Science Advances 6(18). DOI: [10.1126/sciadv.aaz8031](https://doi.org/10.1126/sciadv.aaz8031)

Copyright: Content may be subjected to copyright.